BIODELIGNIFICATION OF OIL PALM TRUNK- OPTIMIZATION

HAZIATUN BT. MOHD FAZI

A thesis submitted in partial fulfillment of the requirements

for the award of the degree of

Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering

Universiti Malaysia Pahang

January 2012

ABSTRACT

Biodelignification is a process to remove lignin from the lignocellulosic materials to obtain celluloses. Hence this study is introduced to study the optimum value of the most affected parameters to the biodelignification process in order to maximize the removal of the lignin. Those parameters are temperature, pH, moisture content and also fungi to medium ratio. As for this research, a local type of fungi, *Pleurotus ostreatus* or commercially known as 'Oyster Mushrooms', were used as an agent of delignification and the raw material chosen are oil palm trunk wastes .In order to optimize conditions for production of lignocellulolytic enzymes by the oyster mushroom, a Response Surface Methodology (RSM) based experiment was designed by using Design Expert software. In this research, 30 runs of experiment were done. After obtaining the experimental design table, the experimental procedures were preceded and the values of the parameters were set according to the table at respective value. After that, the lignin content was analyzed by using Klason Lignin Method. With this method, the lignin content can be determined for various values of parameters. Then, the results obtained were analyzed again by using Design Expert software. At the end of this research, the optimum conditions obtained for the lignin degradation of oil palm trunk fiber are with temperature of 25 °C, pH value of 7.0, moisture content of 0.21 ml and fungi to medium ratio of 2.5 with the amount of predicted lignin degraded is 15.37%. Finally, the conformational test was done with the optimum conditions obtained and the error between the actual and predicted value is 18.7%. However, for better results, the center point in RSM should be tested before designing the experiment.

TABLE OF CONTENTS

		Page
SUPERVISOR'S DECLARATION		i
STUDENT'S DECLARATION		ii
DEDICATION		iii
ACKNOWLEDGEMENT		iv
ABSTRACT		v
ABSTRAK		vi
TABLE OF CONTENT		vii-x
LIST OF TABLES		xi
LIST OF FIGURES		xii-xiii
LIST OF SYMBOLS		xiv
LIST OFABBREVIATIONS		xv
LIST OF APPENDICES		xvi
CHAPTER 1	**INTRODUCTION**	
1.1	Background of Study	1
1.2	Problem Statement	2
1.3	Research Objective	2

1.4	Scope of Research	3
1.5	Rationale and Significant	3
CHAPTER 2	**LITERATURE REVIEW**	
2.1	Biodelignification	4
	2.1.1 Cellulose	5-6
	2.1.2 Application of Biodelignification	6-7
2.2	Raw Material (Lignocellulosic Material)	7
	2.2.1 Banana Stem Waste	8
	2.2.2 Corn Stalk	8-9
	2.2.3 Oil Palm Trunk Waste	9-10
	2.2.4 Selection of Raw Material Used	10-11
2.3	Methods of Biodelignification	11-12
	2.3.1 Soft-rot Fungi	12
	2.3.2 Brown-rot Fungi	12-13
	2.3.3 White-rot Fungi	13
	2.3.3.1 *Pleurotus Ostreatus* (Oyster Mushroom)	14
	2.3.3.2 *Phanerochaete Chrysosporium*	14-15
	2.3.4 Selection of Method of Biodelignification	15
2.4	Optimization	
	2.4.1 Factors	
	2.4.1.1 Temperature	16
	2.4.1.2 pH	16-17
	2.4.1.3 Moisture Content	17
	2.4.1.4 Fungi to Medium Ratio	17

2.4.2 Response Surface Methodology (RSM) 17-18

2.5 Conclusion 18

CHAPTER 3 RESEARCH METHODOLOGY

3.1 Process Flow Chart 19-20

3.2 Response Surface Methodology (RSM) 20-21

3.3 Oil Palm Trunk Delignification Preparation 22

3.3.1 Temperature 22

3.3.2 pH Value 22-23

3.3.3 Moisture Content 23

3.3.4 Fungi to Medium Ratio 23

3.4 Klason Lignin 23-24

3.5 Final Procedures 24

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Factors in Biodelignification Process 25-26

4.1.1 Effect of Temperature 26-27

4.1.2 Effect of pH Value 27

4.1.3 Effect of Moisture Content 28

4.1.4 Effect of Fungi to Medium Ratio 28-29

4.1.5 Interaction between Factors 29-34

4.2	Analysis of Lignin Degraded by Klason Lignin	35-38
4.3	Analysis using Response Surface Methodology (RSM)	38-40
4.4	Optimum Condition	41

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion	42
5.2 Recommendation	43
REFERENCES	44-48
APPENDICES	49-52

LIST OF TABLES

Table No.	Title	Page
2.1	Chemical composition of different parts of the oil palm	10
2.2	Composition of various types of lignocellulosic-biomass materials (% dry weight)	11
3.1	Ranges of optimization for all factors	20
3.2	Experimental Design Table	21
4.1	Percentage of lignin left	35
4.2	Percent losses of different components of the hardwood and softwood pretreated with *Enchinodontium Taxodii*	36
4.3	Comparison between actual value and predicted values	40
4.4	Comparison of predicted and actual value of optimization	41
A1	Quantity of Na_2HPO_4 and citric acid required for various pH	49

LIST OF FIGURES

Figure No.	Title	Page
2.1	Structure of cellulose	6
2.2	Building blocks of lignin	6
3.1	Process flow chart	19
4.1	Perturbation plot	25
4.2	The interaction between Factor A, temperature and C, moisture content	30
4.3	The interaction between Factor A, temperature and B, pH value	31
4.4	The interaction between Factor B, pH value and D, fungi to medium ratio	31
4.5	The interaction between Factor A, temperature and D, fungi to medium ratio	32
4.6	The interaction between Factor C, moisture content and D, fungi to medium ratio	33
4.7	The interaction between Factor B, pH value and Factor C, moisture content	34
A1	Citic acid (0.1M)	50
A2	Na_2HPO_4 (0.2 M)	50

A3	Reactor for the biodelignification process	51
A4	Shaking water bath for Klason Lignin	51
A5	Laminar flow hood for transferring fungi into the reactor	52

LIST OF SYMBOLS

g	Gram
ml	Milliliter
°C	Degree celsius
m^2/g	Squared meter per gram
w/w	Weight over weight
%	Percentage
μm	Micrometer

LIST OF ABBREVIATIONS

AFEX	Ammonia fiber explosion
ANOVA	Analysis of Variance
CCD	Central Composite Design
CcP	Cytochrome c peroxidase
CMCase	Endo-1,4-β-D-glucanase
CO_2	Carbon dioxide
HRP	Corseradish peroxidase
LHW	Liquid hot water
RSM	Response Surface Method
YMPG	Yeast-malt-peptone-glucose

LIST OF APPENDICES

Appendix	Title	Page
A	Materials and Methodology	49-52

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is one of the countries in Asia that practiced agriculture as one of its major industries of economic importance. Hence there are a lot of plantations in Malaysia especially oil palm tree and Malaysia is one of the palm oil producers around the world. Oil palm tree will start bearing fruits after 30 months of field planting and will continue to be productive for the next 20 to 30 years, thus ensuring a consistent supply of oils. After about 30 years, all of the palm tree will be felled and the trunks will be disposed. Aside from just disposing it, there will be a lot of advantages if it is processed and used in many industrial uses. This is because oil palm trunk is one of the hardwoods that rich in cellulose composition which known as lignocellulosic biomass.

The lignocellulosic biomass, which represents the largest renewable reservoir of potentially fermentable carbohydrates on earth, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Lignocellulosic wastes such as oil palm trunk wastes contain cellulose, hemicelluloses and lignin. Cellulose in these wastes is very useful in many industrial purposes especially in production of bioethanol.

To remove the lignin from the waste, a method of delignification can be used. There are various methods used in the pretreatment of lignocellulosic wastes which are chemical, physicochemical, physical, and biological pretreatment and a wide variety of species are involved.

1.2 Problem Statement

Cellulose is the substance that makes up most of a plant's cell walls besides hemicellulose. Since it is made by all plants, it is probably the most abundant organic compound on earth. Aside from being the primary building material for plants, cellulose has many others uses. According to how it is treated, cellulose can be used to make paper, film, explosives, and plastics, in addition to have many other industrial uses. Those cellulose and hemicelluloses can be obtained from agricultural wastes such as oil palm trunk waste by using biodelignification. However, in past decades, the palm oil residues such as oil palm shells, mesocarp fibers and empty fruit bunch and oil palm fronds and oil palm trunk became the most abundant biomass resources in spite of Malaysia is one of the biggest producers of oil palm tree in the world. Hence, aside from disposing the wastes, the biodelignification is a method to produce more cellulose. In this process, the usage of fungi as an agent of delignification seems to be an effective method because even though it does consume a lot of time in order to remove lignin from the oil palm trunk, the energy consumption is lower and less cost compared to other pretreatment methods.

1.3 Research Objective

The main purpose of this research is

i. To study the optimization of biodelignification of oil palm trunk waste.

1.4 Scope of Research

The scopes of this study are

i. To use a type of local fungi (*Pleurotus ostreatus*) as an agent of delignification process.
ii. To optimize four factors which affect the lignin removal the most which are temperature, pH, moisture and fungi to medium ratio by using Response Surface Methodology (RSM).
iii. To determine the lignin content of the oil palm trunk using Klason Lignin method.

1.5 Rationale and Significant

By doing this study, the optimum condition for biodelignification of oil palm trunk can be determined including four factors which are temperature, pH, moisture content and fungi to medium ratio. Hence, the quantity of the cellulose obtained can be maximized when the optimum conditions are successfully determined. The process also can be done with a large amount due to the large quantity of raw materials that can be found nowadays and may produce more cellulose. However, the cost of the production can be decreased

CHAPTER 2

LITERATURE REVIEW

2.1 Biodelignification

Biodelignification is delignification process by using any biological method where delignification means the removal of the lignin from the wood by any processes. Lignin decomposition is one of the processes for delignification and it is actually a slow process whether in aerobic or anaerobic state. Biodegradation of cellulosic crop residues, agricultural solid wastes and municipal solid wastes in the absence of oxygen can be used to reduce pollution and produce biomethane as a fuel (Romana *et al.,* 2000). Common for all types of pretreatment is that they should minimize loss of sugars, consume a minimum of energy, and increase enzymatic digestibility. This is done by removing or rearranging the lignin structure, partial or full removal of hemicellulose from the fibers, and possibly altering the cellulose structure (Mette *et al.*, 2009). A variety of biological treatment is available for delignification from fungi treatment to the usage of other microbiological strains. The results obtained from a research done by Maria *et al.*, 2005 showed that the tested fungi were effectively involved in humification and lignin degradation of horticultural wastes and might be used as inoculants in a pre-treatment process before composting in order to reduce the resistance of the substrate to biodegradation.

2.1.1 Cellulose

Cellulose is a major component of the plant cell wall and different cellulose synthases perform cellulose synthesis in the primary cell wall and the secondary cell wall (Saxena and Malcolm, 2007)**.** Cellulose is a simple polymer, but it forms insoluble, crystalline microfibrils, which are highly resistant to enzymatic hydrolysis (Pierre and Jean-Paul, 1994). One of the studies from Howard *et al*. (2003) shows that the cellulose composition in the hardwood stem and softwood stem is the higher than hemicelluloses and lignin. The application of the cellulose as a precursor for chemical modifications was exploited extensively even before its polymeric nature was determined and well understood. That is why the cellulose is needed in industry. Cellulose is one of the most abundant biopolymers on earth. It exists in nature as fibrils, called microfibrils, which are only a few nanometers in diameter. Hemicellulose and lignin surround the cellulose microfibrils in plant cell walls (Masahisa *et al.*, 2010). In industry, the recovery of glucose from lignocellulosic biomass has been regarded as a powerful method to obtain valuable products such as bioethanol and various chemicals, without competing with food production (Masakazu *et al*., 2010). These products are presumably converted into true inducers by transglycosylation reactions. Several applications of cellulases or hemicellulases are being developed for textile, food, and paper pulp processing. These applications are based on the modification of cellulose and hemicellulose by partial hydrolysis. Total hydrolysis of cellulose into glucose, which could be fermented into ethanol, isopropanol or butanol, is not yet economically feasible. It is especially the rapid enzymatic decomposition of cellulose that is a key issue in the efficient recovery of glucose (Pierre and Jean, 1994).

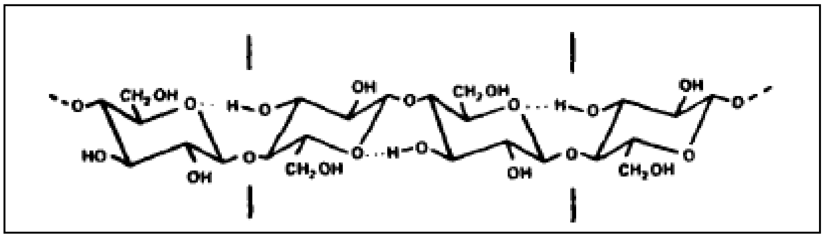

Figure 2.1: Structure of cellulose

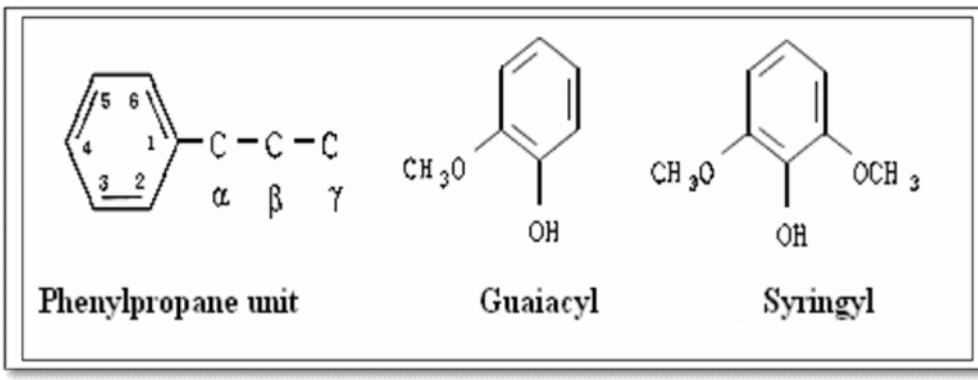

Figure 2.2: Building blocks of lignin

Source: James and Jeffrey (1997)

2.1.2 Application of Biodelignification

In the early years of application of the biological method various type of fungi such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Schurz, 1978). Though these fungi depend on cellulose to survive, the brown-rots mainly attack cellulose alone, ignoring the lignin. The other white- and soft-rots attack both cellulose and lignin during the fungi growth.

The application of fungi in the biological method of delignification process will also likely to produce other enzymes or cellulases that can be used for the next sequencing process in the production of bioethanol. Boominathan and Reddy

(1992) discovered that white-rot fungi *P. Chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation.

Other white-rot fungi also proved that these enzymes were produce during the delignification process and their presence improved the cellulose hydrolysis process (Kirk and Farrell, 1987; Waldner *et al*., 1988). Despite the benefits of the biological method such as the enzymes or cellulases that were produced and the low energy and economy cost, the biological method has a low rate of hydrolysis if it is to be used as a pretreatment process in the bioethanol production.

2.2 Raw Material (Lignocellulosic Material)

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose (Betts *et al*., 1991). Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). There are a lot of studies that has been done on the lignocellulosic materials. Lignocellulosic materials have two different types of surface area which is external and internal. The external surface area is related to the size and shape of the particles, while the internal surface area depends on the capillary structure of cellulosic fibers. Typically, dry cellulosic fibers have small size, about 15 to 40 µm, and therefore they possess a considerable external specific surface area, for example, 0.6-1.6 m^2/g (Taherzadeh and Karimi, 2008).

2.2.1 Banana Stem Waste

Banana is one of the important fruit and vegetable crop plants and the well-known species are abaca (*Musa Textilis*) and other wild banana plants used as a source of fibres for the paper and cordage industry (Franco *et al.,*1993). Banana plant is lignocellulosic which contain cellulose, hemicelluloses and lignin. Banana stem waste commonly contain of 41% - 57% cellulose, 8% - 12% hemicelluloses and 24% - 27% lignin (Scurlock *et al.,* 2004; Silveira *et al.*, 2007). Banana stem waste has a high organic content (83%); with 15–20% (w/w) lignin and cellulose which gives it a sheath-like texture (Kalia *et al*., 2000). The separation of lignin from lignocellulosic biomass by organic solvents can be considered as a very effective approach. According to Marcelle *et al*., (2002) the pulping process using formic acid/acetic acid/water to selectively separate cellulose, hemicellulose and lignin from banana stem at atmospheric pressure was optimised. The main residual wastes of the banana crop are leaves and pseudostems, both containing high levels of lignocellulose. These lignocellulose materials are efficient substrates for white-rot fungi, which produces lignolytic and cellulolytic enzymes that have numerous applications in industrial processes for food, drug, textile and dye use (Reddy *et al.*, 2003). However, banana stem has low lignin content and makes the delignification of banana stems appears difficult (Marcelle *et al.,* 2002).

2.2.2 Corn Stalks

Cellulose gives plants structure, and cell walls of plant cells are cellulose. Cellulose is the basis of wood, grass, and stalks of plants, and makes up 44% of all biomass. Cellulose is a structural entity, so its purpose is to provide the plant with stability. According to research published in the online edition of the American Chemical Society journal, Environmental Science and Technology by scientists at Rice University, when corn stalks and stover are going to be used for ethanol production, using less nitrogen produces a better quality feedstock. Various kinds of solid materials containing lignin were obtained by fractionation (autohydrolysis and organosolvolysis) of corn (*Zea mays*) stalks (Manuel *et al.,* 1999). Corn Stalks remaining after it was harvested contain 43% polysaccharides consisting mainly of hemicellulose and cellulose, 29% lignin, 7% ligin, 5% ash, and 16% others (Chahal *et al*., 1990).

2.2.3 Oil Palm Trunk Waste

Oil palm (*Elaeis guineensis*) for palm oil production needs to be replanted at an interval of 20 to 25 years in order to maintain oil productivity. Oil palm trunk is categorized as hardwood. Oil palm tree has been planted since 20-30 years ago and now is the time for the replantation. That is why the amount of waste for this high processing value is estimated to be around 33 million tonnes including empty fruit bunches, oil palm trunks, fibers and shells (IMPOB, 2009b; Mohamed and Lee, 2006). In 2007, as much as 10, 827 tonnes of oil palm trunks are obtained as waste showing that these oil palm trunks are the largest contributors in waste from the agricultural industry (Goh *et al*., 2009). Like any other wood structure, hardwood is categorized under lignocellulose. The trunk, leaves and shell of the oil palm tree are lignocellulosic materials. Another study also state that oil palm is a lignocellulosic material which rich in carbohydrates in the form of starch and sugar and containing cellulose, hemicelluloses and lignin (Murai *et al*., 2009). It is an abundant waste material at replantation and harvesting sites in Malaysia and in many parts of South East Asia (Sreekala *et al.,* 1997). Large quantities of this waste are left in the field as underutilized resources. Oil palm is now considered to be one of the most promising non-wood lignocellulosic raw materials for various types of wood-based panels (Sulaiman *et al*,. 2009). All of this parts yield cellulose and monosugars such as glucose, xylose and arabinose after pretreatment. By microbial fermentation, these sugars could be used as substrate to produce renewable energy such as ethanol and hydrogen. Oil palm trunk is made up of three main structural components which are cellulose 34.5%, hemicellulose (mainly xylan) 31.8% and lignin 25.7%. A study done by RunChang and Tomkinson (2001) stated that the chemical composition (% dry weight, w/w) of oil palm trunk fiber is the following: cellulose 41.2%, hemicelluloses 34.4%, lignin 17.1%, ash 3.4%, extractives 0.5%, and ethanol soluble 2.3%.

Table 2.1: Chemical Composition of Different Parts of the Oil Palm

Parts of oil palm	**Extractives**	**Chemical Composition (%)**		
		Holocellulose	**Alpha Cellulose**	**Lignin**
Bark	10.00	77.82	18.87	21.85
Leaves	20.60	47.7	44.53	27.35
Frond	3.50	83.13	47.76	20.15
Mid-part of trunk	14.50	72.6	50.21	20.15
Core-part of trunk	9.10	50.73	43.06	22.75
Frond	1.40	82.2	47.60	15.20
Trunk	5.35	73.06	41.02	24.51
Kenaf	-	82	-	-
Hardwood	0.1-7.7	71-89	31-64	14-34
Softwood	0.2-8.5	60-80	30-60	21-37

Source: Rokiah *et al.* (2011)

2.2.4 Selection of Raw Material Used

Most of the previous researches did not used hardwood as a source of raw material. Softwood was chose over hardwood for most of these tests mainly because it has a much weaker structure compared to hardwood. Cellulose and hemicellulose contents are more in hardwoods (78.8%) than softwoods (70.3%), but lignin is more in softwoods (29.2%) than hardwoods (21.7%)(Balat, 2009). That is why the hardwood is chosen to obtain more cellulose. Hence, for the selection of the raw material used, oil palm trunk wastes are chosen because there are a lot of oil palm trunk residues available in this country and making it as the most suitable raw material for this process compared to corn stalks and banana stem wastes. The structural composition of various types of lignocellulosic-biomass materials are given in Table 2.2 (Demirbas, 2005).

Table 2.2: Composition of various types of lignocellulosic-biomass Materials (% dry weight)

Material	Cellulose	Hemicelluloses	Lignin	Ash	Extractives
Algae(green)	20-40	20-50	-	-	-
Cotton, flax, etc.	80-95	5-20	-	-	-
Grasses	25-40	25-50	10-30	-	-
Hardwoods	45±2	30±5	20±4	0.6±0.2	5±3
Harwood barks	22-40	20-38	30-55	0.8±0.2	6±2
Softwoods	42±2	27±2	28±3	0.5±0.1	3±2
Softwood barks	18-38	15-33	30-60	0.8±0.2	4±2
Cornstalks	39-47	26-31	3-5	12-16	1-3
Wheat straw	37-41	27-32	13-15	11-14	7±2
Newspapers	40-55	25-40	18-30	-	-
Chemical pulps	60-80	20-30	2-10	-	-

2.3 Methods of Biodelignification

A large number of microorganisms have attracted particular attention for their potential ability of lignin degradation (Hatakka, 2001). In the early years of application of the biological method various type of fungi such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicelluloses in waste materials (Schurz, 1978). White-rot fungi are known as the most efficient lignin degraders, in which the representative species *Phanerochaete chrysosporium* has been most extensively studied due to the ability to degrade a wide range of organic substrates (Wen *et al*., 2009; Yu *et al*., 2009). Fungi are able to break down resistant materials such as cellulose, gums, and lignin. They dominate in acidic, sandy soils and in fresh organic matter. In natural decomposition of lignocellulosic matter, both fungi and aerobic bacteria play an important role in degrading holocellulose and lignin to lower-molecular-weight products, some of which are then further metabolized by facultative and obligate anaerobic soil bacteria and actinomycetes (Maccubbin and Hodson, 1980). Biological pretreatment involves microorganisms such as brown-, white- and soft-rot fungi that are used to degrade lignin and solubilize hemicellulose. Among the three types of the fungi, white-rot fungi are the most effective biological pretreatment of lignocellulosic materials (Taherzadeh, 2008). The advantages of biological pretreatment include low energy requirement and mild environmental conditions.

2.3.1 Soft-rot Fungi

In soft-rot decay mainly cellulose and hemicelluloses are degraded, while lignin degradation is restricted. Soft-rot is characteristic to wood in wet environments such as railway slippers, poles, piles, ship and boat wood. In buildings soft-rot is found in moist window frames. Both softwoods and hardwoods are attacked by soft-rot fungi. Characteristic species are *Chaetomium Globosum, Richurus Spiralis* and *Phialophora Mutabilis* (Schultz *et al*., 2003).

2.3.2 Brown-rot Fungi

Brown-rot fungi utilize carbohydrates and lignin only slightly affected (Fan *et al.,* 1987). Brown-rot fungi produced high levels of hydrolytic activities and no phenoloxidase activity. An example of brown-rot fungi, *Laetiporeus sulfurous* demonstrated a very limited degradative capacity, contrasting with *Wolfiporia cocos,* which induced an effective decay. However, the fungi providing the highest values of lignin loss were also responsible for the highest values of polyoses removal (Angela and Ferraz, 2001). Brown-rot is a type of wood decay